Message

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Sent: 9/11/2019 1:13:44 PM
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Subject: RE: chloroprene -- human lung

From previous times I checked with Matt, I think he'd have trouble remembering this derivation from 15+ years ago, and really we'd need to see the code for the in vitro model to know for sure what was done.

But I checked and the data in Figure 7 have an initial decay rate corresponding to kf = 4/h. Below is an overlay plot (excel plot with clear background on top of image from the paper), blue dots are exponential decay with a rate constant of 4/h; this is the value that matches the data. The rest depends on how one defines the model, what units are used to normalize this observed rate and split it into separate constants, with the fact that the experiments were run at 1 mg/mL CSP.

Dividing this by a cytosolic protein concentration of 1000 mg/L (note I've switched from /mL to /L) yields an effective $ks*C^{BS(0)}$ = 0.004 L/h/mg protein. This is numerically identical to the value in the last column of Table 4, based on the assumption that clearance is proportional to the concentration of enzyme in solultion:

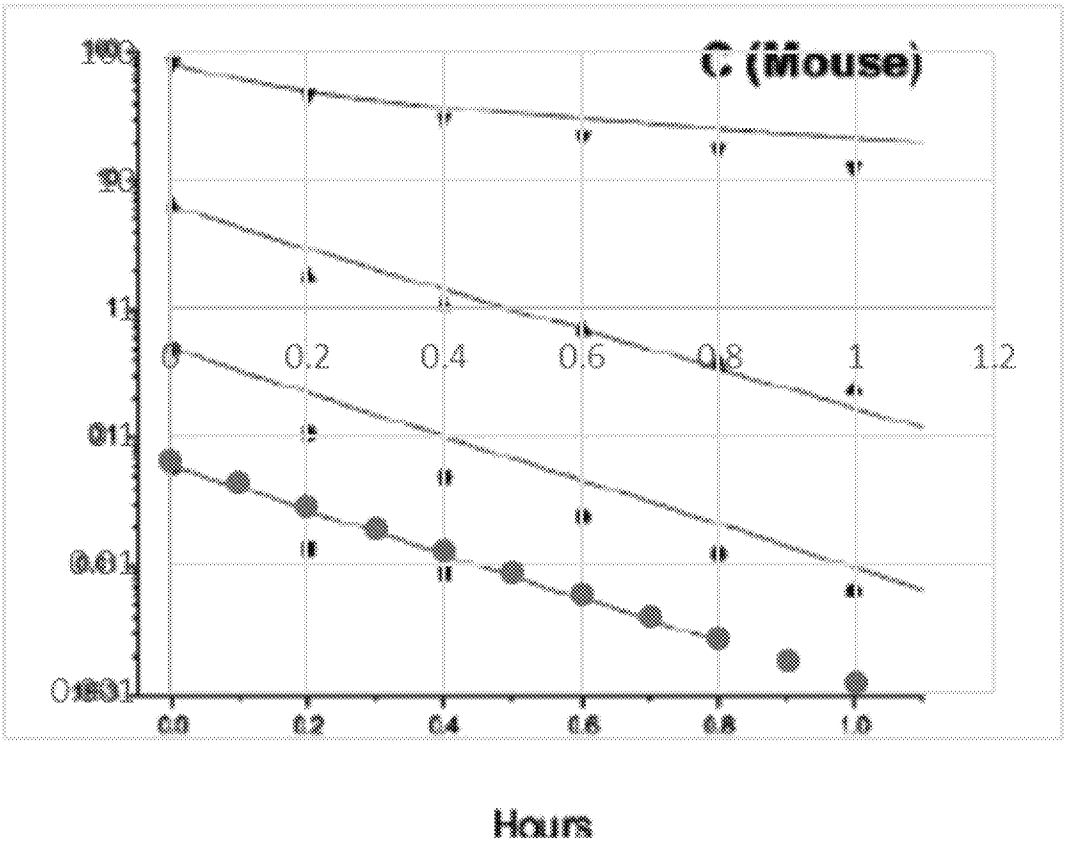
Rate (umol/L/h) = Cceo (uM) x (0.004 L/h/mg protein) x Ccsp (mg protein/L)

So this is the correct units (L/h/mg) for the combined constant. (Or it can be converted to 4 mL/h/mg protein.)

Taking ks to be an intrinsic binary constant L/umol/h, it follows the units of C^{BS} are (L/h/mg protein)/(L/umol/h) = umol/mg protein, micromoles of binding site per mg cytosolic protein.

But the exact units of each component don't matter for evaluating clearance in liver or lung tissue at low concentrations, where we can use the ~ first-order term.

-Paul



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Subject: RE: chloroprene -- human lung

Hi Paul

It looks like the footnote to Table 4 is incorrect. The units of the concentration of protein binding sites ($C^{BS(0)}$) should be umol/L/(mg cytosolic protein), and the units of the second order reaction ($ks*C^{BS(0)}$) should be 1/hr/(mg cytososolic protein)^2. Both parameters were estimated from the data in Figure 7. If they doubled the mg protein in the same volume of media, they would have observed twice the rate of metabolism, but 1 mg was used throughout the study.

With kind regards
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Subject: RE: chloroprene -- human lung

Harvey, all,

I have been reviewing the results in Himmelstein et al. (2004) (1st paper), trying to interpret what they tell us about the relative rate of metabolism of the oxidative metabolite, 1-CEO, in humans vs. rodents. I am having trouble with the units of values shown in Table 4, and how these results would translate to in vivo. The table and footnotes are copied below.

One thing that's clear from the paper's text is that the units of the product $ks \cdot C^{BS(0)}$ should be 1/h/mg protein or $(h^{-1})(mg\ protein)^{-1}$, not h/mg/protein (if other units are assumed correct for now).

Now based on these units if I conduct an incubation in 1 mL solution with 1 mg protein with Cceo (uM) concentration of 1-CEO, then the rate of metabolism would be

Cceo (uM) x $ks \cdot C^{BS(0)}$ (1/h/mg protein) x (1 mg protein) [=] uM/h,

Where uM = umole/liter and "[=]" means "has units of". So the result is the rate of concentration reduction in the incubation.

Now suppose I double the volume to 2 mL and double the amount of protein to 2 mg, keep the GSH and 1-CEO concentration the same?

Well, according to the equation above, the rate would then be:

Cceo (uM) x $ks^* C^{BS(0)}$ (1/h/mg protein) x (2 mg protein); i.e., the concentration loss rate would double. But how would the rate double when I've kept the ***concentration*** of all components the same, just doubled the incubation volume? Presumably, the rate of concentration loss in the first mL is the same as the rate of concentration loss in the 2nd mL, both of which are identical to the 1 mL experiment.

If I use the protein concentration for the incubation (mg protein/mL) in the equation, then the resulting units would be uM/h/mL, which still implies that the concentration loss rate is proportional to total volume, which doesn't make sense. Concentration loss rate is the loss per unit volume, it should be independent of total system volume.

So I can only conclude that the normalization of $ks \cdot C^{BS(0)}$ should actually be per protein concentration (mg protein/**mL of solution**); i.e., $ks \cdot C^{BS(0)}$ has units of mL/h/mg protein. Then I would use the concentration of cytosolic protein (mg/mL) to calculate the rate:

Cceo (uM) x $ks \cdot C^{BS(0)}$ (mL/h/mg protein) x (mg protein/mL) [=] uM/h.

If that is correct, then I don't predict a change in concentration loss rate if I just double the incubation volume.

Why this matters: IVIVE. If my interpretation is correct, and mCSP is the ***concentration*** of cytosolic protein in the liver (mg/g liver), then

$ks \cdot C^{BS(0)}$ (mL/h/mg protein) x mCSP (mg protein/g liver) [=] mL/h/g liver; i.e., a clearance per gram of liver. This makes sense to me. To get clearance in the liver as a whole I'd jst multiply by the total liver mass (g). Does this make sense?

-Paul

TABLE 4

Optimized Parameters for Cytosolic Glutathione *S*-transferase Activity toward (1-chloroethenyl)oxirane (1-CEO)

		Activity of cytosolic glutathione *S*-transferase[a]		
Tissue	Species	ks	$C^{IRS(0)}$	ks · $C^{IRS(0)}$
Liver	Mouse	0.0015	2.7	0.0040
	Fischer rat	0.0074	0.92	0.0068
	Wistar rat	0.011	0.56	0.0063
	Hamster	0.024	0.54	0.0130
	Human	0.0017	0.94	0.0016
Lung	Mouse	0.0011	2.01	0.0022
	Fischer rat	0.0023	0.70	0.0016
	Wistar rat	0.0051	0.18	0.00092
	Hamster	0.015	0.038	0.00056
	Human	0.0028	0.44	0.0012

Note. ks (l/μmol/h/mg cytosolic protein), rate constant $C^{IRS(0)}$ (μmol/l) as initial concentration of protein binding sites and ks · $C^{IRS(0)}$ (h/mg protein) describing enzymatic 1-CEO-GSH conjugate formation as a pseudo-second order reaction.

[a]First order reaction of 1-CEO with GSH was measured as kf = 0.07 h^{-1} independent of protein.

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Subject: RE: chloroprene -- human lung

Hi Paul

I checked with Miyoung and the human values in Table 3 are based on Viera et al. (1998) Table 1, which provides data from a single adult subject.

With kind regards
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Subject: RE: chloroprene -- human lung

Thanks, Harvey.

The pool size could be bigger, but it is good support that the Lorenz data don't under-estimate human lung activity. … On the other hand, how could I forget?! There is this paper from Miyoung, attached. The ratio shown in table 3 is 0.9%, or 0.009. It is citing

Vieira, I., Pasanen, M., Raunio, H., and Cresteil, T. 1998. Expression of CYP2E1 in human lung and kidney during development and in full-term placenta: A differential methylation of the gene is involved in the regulation process. Pharmacol. Toxicol. 83:183–187, which in turn cites a 1996 article describing the initial tissue collection. Both are also attached, sorry about the rotation of the 1st page of the '98 paper, it's how it is on HERO.

From the '96 paper: Adult liver samples were obtained from donors for kidney transplantation. Donors had no severe chronic pathology and had generally died from a traffic accident. They
had no re-peated drug consumption. No information was available regarding their smoking and drinking habits.

Some plots in the '96 paper indicate 14 donors, others 10 donors.

-Paul

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Hi Paul

This paper provides relative expression ratios across tissues in the human for a variety of cyps.

From Table 1, the ratio of lung/liver expression for 2e1 is 0.0173/53.8 = 0.00032

Adding 2F1 in the lung (which was not detected in the liver), it becomes (0.0173 + 0.0128)/53.8 = 0.00056

That's about a factor of 3 lower than the lung/liver activity ratio from Lorenz (A1 = 0.00143)

This supports the use of A1 derived from Lorenz to estimate human lung metabolism for 2e1 substrates like chloroprene or methylene chloride as a conservative approach.

With kind regards
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Subject: chloroprene -- human lung

Harvey, all,

The Lorenz et al. (1984) paper from which the 'A1' for lung:liver metabolism is calculated use 7-ethoxycoumarin as a substrate, which is not a pure 2E1 substrate, but also metabolized by human 1A2, which would not be relevant for CP, and some others.

https://www.ncbi.nlm.nih.gov/pubmed/8573198
https://www.ncbi.nlm.nih.gov/pubmed/16719387

I didn't look thoroughly, but didn't see that Lorenz gave the concentration of 7-EC they used (the 1st reference above indicates that at high concentrations it's more 2E1-specific), and the paper they cite for the method is a review paper. I stopped at that point.

Also, while Lorenz had data on 13 separate human subjects for liver metabolism and 10 for lung (all non-smokers!), they were all having biopsies or surgery for a reason.

No human data set is ideal, but do you know of a 3rd data set for human lung vs. liver activity we could consider, to triangulate the thing, as it were?

-Paul